

P1-004

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**Role of cytology accompanied by the study of the occurrence of p16INK4a and O6-methylguanine-DNA transferase (MGMT) promoter methylation in the cells of the pleural cavity washings fluid in early diagnostics of non-microcellular lung cancer (NSCLC) metastatic changes into pleura**

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**Background:** Metastases of NSCLC into pleura are a wrong prognostic index. Sometimes, it is possible to detect presence of metastases in pleural effusion before surgical treatment. It is very importance to exclude the risk of surgery in these patients. The aim of the study was to determine whether the washing of pleural cavity can help in early detection of NSCLC metastases into pleura. Additionally aberrant promoter methylation of p16INK4a and MGMT of pleural lavage cells were examined.

**Methods:** Pleural lavage fluid were collected from 32 patients (aged 48 - 77 yrs) operated on NSCLC (studied group) and 6 patients (aged 30 -71 yrs) operated for other reasons (control group). Promoter methylation status was examined in 27 cases by using methylation-specific polymerase chain reaction (MSP). Results of cytological studies and MSP examination were analyzed according to NSCLC histological type, TNM stage and localization of neoplastic foci in pleural.

**Results:** There were 50% patients with I stage NSCLC, 19% with II stage and 31% with III stage. Squamous cell carcinoma was diagnosed in 13 cases, adenocarcinoma in 14 and large cell carcinoma in 3 cases. Presence of neoplastic infiltrations into visceral pleura was detected by histological examination in 34% patients and parietal pleural in 16% patients. Cytological results of pleural cavity washing fluid were positives in 4 cases, negatives in 21 cases and doubtful in 7 cases. Aberrant methylation of p16INK4a and MGMT genes was discovered in 22 patients. There were not significance differences between presence of neoplastic cells in lavage fluid and type of cancer, TNM staging or pleural cancer infiltration. Aberrant methylation was more frequent in studied group (p=0,0003). There were not dependence between aberrant methylation and type of cancer, advanced TNM staging, presence of pleural neoplastic foci.

**Conclusions:** Cytology and analysis of p16INK4a and MGMT methylation of pleural cavity washings fluid seem to be a promising examinations in early detection of NSCLC metastases into pleura.

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**Tissue content of Survivin mRNA and Survivin Expression in nonsmall cell lung cancer for diagnosis and prognosis.**

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**Background:** Deregulation of apoptosis pathway is one of crucial mechanisms causing tumorigenesis and driving cancer progression. Survivin is a member of the inhibitor of apoptosis, IAP gene family. It plays a dual role in both cell death suppression and cell cycle regulation at the G2/M phase. Its expression also contributes to resistance to chemo- and radiotherapy of the cancer cells.

**Objectives:** This study was aimed at determination of surviving mRNA expression semiquantitatively comparing to the surviving expression in tumor tissue of the nonsmall cell lung cancer. Correlation between survivin mRNA content and expression of the commercially available survivin in the tumor tissue and pathological parameters is also evaluated proving the significance of survivin in cancer diagnosis and prognosis.

**Materials and Methods:** 22 cases of surgically resected nonsmall cell lung cancer were included during the year 2005 and the early 2006. Total RNA was extracted from fresh frozen tissues of both cancer and corresponding normal lung tissues. The formalin-fixed, paraffin-embedded cancer tissue has been tested with commercially available survivin antibody immunohistochemically. The correlation between mRNA expression and protein expression was evaluated with pathological parameters using Chi-square test.

**Conclusions:** Selective survivin expression in the lung cancer tissue not in the normal lung tissue makes it a potential diagnostic marker however, the prognostic marker to select the cases that might be chemoresponsive needs to verify with the other markers.

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**Development of a candidate tumor marker against human lung cancer**

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**Background:** We used the differential display RT-PCR method using various types of normal human lung tissues, lung cancer cell line, and lung cancer tissues and then identified three hundred unique oncogenes overexpressed in human lung cancer tissues. The objective of this study was to investigate the significance of these newly identified oncogenes as candidate tumor markers for the human lung cancers.

**Methods:** Northern blot analysis showed that identified three hundred oncogenes were overexpressed in human lung cancer tissues compared to normal lung tissues, respectively. Recombinant oncoproteins were overproduced in E. coli and purified. Using these as immunogens, more than 100 monoclonal antibodies and each rabbit polysera were generated. Tumorous tissues and their corresponding non-tumorous tissues from cancer patients who underwent surgical resection were examined for the expression of each new oncoprotein using immunohistochemical staining and Western blot, respectively.

**Results:** Each polyclonal antibody and monoclonal antibody reacted strongly and specifically with new respective oncoprotein was used for the biochemical and immunohistochemical studies. The expressions of each new oncoprotein were significantly observed in the tumorous portion of human lung cancers.

**Conclusions:** Our study suggests that newly developed cancer-specific antibodies will facilitate the assessment of human lung cancers in tumor diagnostic histopathology and may be useful as specific tumor markers against human lung cancers.